Reference: *Biol. Bull.* **183**: 233–241. (October, 1992)

Is There Extraovarian Synthesis of Vitellogenin in Penaeid Shrimp?

M. FAINZILBER[1], M. TOM[1], S. SHAFIR[1], S. W. APPLEBAUM[2], AND E. LUBZENS[1,*]

[1]*Israel Oceanographic and Limnological Research, P.O. Box 8030, Haifa 31080, Israel, and*
[2]*Department of Entomology, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel*

Abstract. Extraovarian synthesis of vitellogenin (Vg), has been reported for several crustaceans, mainly in the subepidermal adipose tissue (SAT) or the hepatopancreas (HEP). The precise site(s) of Vg synthesis in penaeid shrimp is hitherto unknown and was investigated in a large local species *Penaeus semisulcatus* de Haan. Protein synthesis was determined in SAT and HEP tissue pieces incubated *in vitro*. Incubations were at 25°C for eight hours in an oxygen enriched atmosphere, under sterile conditions in a physiological medium, containing ^{14}C-leucine. At the end of the incubation period, tissue homogenates and medium samples were analyzed for *de novo* protein synthesis. Total protein synthesis was determined by trichloroacetic acid precipitation. Specific vitellin (Vt) synthesis was determined by radioimmunoprecipitation with a polyclonal Vt-specific antiserum. Characterization of other *de novo* synthesized proteins was carried out by fluorography from polyacrylamide gels. Subepidermal adipose tissues removed from females at all stages of ovarian development did not synthesize Vt-specific proteins, in spite of the fact that total protein synthesis levels were high. The major protein synthesized *de novo* in the SAT of males and females is a protein with an identical electrophoretic mobility as hemocyanin in polyacrylamide gels. *In vitro* protein synthesis in HEP tissues was low compared to SAT or ovary systems. Vt-specific *de novo* synthesized protein was identified in HEP's from early vitellogenic females, but constituted less than 15% of total protein synthesis. We have previously shown that ovarian tissues from vitellogenic females incubated *in vitro* exhibited high levels of protein synthesis, an average of 38% of which is Vt-specific (Browdy *et al.*, 1990, *J. Exp. Zool.* **255**: 205–215). The calculated Vt synthesis rates in ovaries were up to 23 times higher than in HEP. We conclude that the extraovarian contribution to vitellogenesis in *P. semisulcatus* is low.

Received 26 August 1991; accepted 1 June 1992.
* To whom correspondence should be sent.

Introduction

Vitellogenesis is associated with the formation of yolk globuli within the developing oocytes in the ovary. Proteins and lipids are the main constituents of yolk, and vitellin (Vt) is the major protein that accumulates within the ovary during this process (Adiyodi and Subramoniam, 1983; Meusy and Payen, 1988). Vitellogenesis is an endocrine-regulated process, and is therefore of interest as a central part of the female reproductive cycle (Quackenbush, 1986; Fingerman, 1987; Charniaux-Cotton and Payen, 1988). It has been intensively studied in the lower vertebrates (Tata, 1976; Lam *et al.*, 1978; Huang *et al.*, 1979) and in various groups of insects (Kanost *et al.*, 1990). There has been increasing interest in crustacean vitellogenesis, due to the growing commercial importance of crustaceans in aquaculture and the interest in new models for basic research (Laufer and Downer, 1988). A critical prerequisite for studying vitellogenesis at the endocrine and cellular levels is identification of the tissues that participate in the synthesis of yolk and its precursors.

The sites of synthesis of Vt or its precursor molecules have not yet been fully established for penaeid shrimps. A protein which reacts immunologically to antiserum prepared against purified Vt was found in the haemolymph of vitellogenic females. This protein, known as vitellogenin (Vg), has been reported in all species studied so far (Kerr, 1968; Ceccaldi, 1970; Wolin *et al.*, 1973; Fyffe and O'Connor, 1974; Caubere *et al.*, 1976; Meusy, 1980; Dehn *et al.*, 1983; Ferrero *et al.*, 1983; Marzari *et al.*, 1986; Tom *et al.*, 1987a; Suzuki, 1987; Nelson *et al.*, 1988; Quackenbush and Keeley, 1988; Quackenbush,

1989a). The presence [illegible] in the haemolymph raises the question of possible [illegible] arian sites for synthesis of Vt or its precursor [illegible].

Previous [illegible] crustacean vitellogenesis have focused on [illegible]s as possible sites for Vg synthesis; ovaries [illegible] ssues, and hepatopancreases (HEP). *De novo* synthesis of Vt in the ovaries has been shown for one isopod (Gohar *et al.*, 1985) and several decapods (Lui and O'Connor, 1976; Eastman-Reks and Fingerman, 1985), including three species of penaeid shrimps (Yano and Chinzei, 1987; Quackenbush, 1989a, b; Browdy *et al.*, 1990). However, there is also convincing proof for extraovarian synthesis of Vg in the adipose tissues of isopods and amphipods (Picaud, 1980; Souty and Picaud, 1981; Junera and Meusy, 1982). Vt-positive immunoreactivity has been reported for the adipose tissue of *Parapenaeus longirostris* (Tom *et al.*, 1987a) and in the HEP of two brachyuran decapods (Paulus and Laufer, 1987). Different groups have reported conflicting results for *in vitro* Vg synthesis by the HEP in penaeids. Yano and Chinzei (1987) and Rankin *et al.* (1989) were unable to show *de novo* Vg synthesis in HEP of *Penaeus japonicus* and *Penaeus vannamei*, respectively. In contrast, Quackenbush (1989a, b) has reported *in vitro* synthesis of proteins immunoreactive with antibodies to yolk in cultures of the HEP of *Penaeus vannamei*. There have been no studies so far on *de novo* synthesis of Vg in penaeid adipose tissues.

Although there is sufficient evidence to support a role for the ovary in Vt synthesis in penaeids, the quantitative contribution of ovarian versus extraovarian synthesis is not clear. We have tried to clarify this question by studying protein synthesis *in vitro* in the three putative vitellogenic tissues of *Penaeus semisulcatus*. We have recently shown that the ovaries of this species synthesize Vt *in vitro*, and that the portion of Vt-specific synthesis from total-protein synthesis varies in accordance with ovarian developmental stages (Browdy *et al.*, 1990). In this report, we summarize our studies on protein and Vg synthesis in the adipose and hepatopancreatic tissues of this marine shrimp species.

Materials and Methods

Chemicals

Amino acids, HEPES, Bis-Tris, vitamins and supplements for media were cell-culture tested grade and were obtained from Sigma. [^{14}C(U)]leucine (308 mCi/m*M*) was purchased from New England Nuclear. All other reagents were of analytical grade.

Seawater

Mediterranean seawater (salinity 40‰) was pumped onshore into a central reservoir and used immediately. For *in vitro* cultures it was filtered through sieves and a 0.45 μm-membrane filter, and finally sterilized by filtering through a heat sterilized 0.22 μm-membrane filter. Dilutions were performed using deionized water (Ionex, Israel) prior to sterilization.

Animals

Adults of *P. semisulcatus* were collected in Haifa Bay, Israel. They were held in 3 m^3 running seawater tanks, at a density of 20 specimens per tank. Water was changed at a rate of 300% per day. Salinity was constant at 40‰, and temperature ranged from 18°C (winter) to 27°C (summer). The animals were fed once a day on a mixture of frozen fish, squid, shrimp, and *Artemia*. Females were individually marked by clipping of the uropods, and ovarian development was monitored externally, according to the methods of Browdy and Samocha (1985).

Polyacrylamide gel electrophoresis (PAGE) and electroblotting

Electrophoresis in non-denaturating conditions was performed according to Davis (1964) in 5% polyacrylamide gels. Coomassie Blue stains for proteins and dithiooxamide stains for copper-conjugated proteins were performed according to Hames and Rickwood (1981).

Western blots from PAGE were done using a Bio-Rad electroblotting apparatus at 400 mA for 4–5 h at 4°C. Proteins were transferred to nitrocellulose papers, dried, and stored at 4°C until staining. Immunostaining was according to Browdy *et al.* (1990).

Purification of Vt and preparation of rabbit specific antiserum

Vitellin was identified as the main constituent of ripe ovaries by comparing the electrophoretic profiles in non-denaturing polyacrylamide gels (PAGE) of ripe and undeveloped ovarian homogenates. It was purified from homogenates of ripe ovaries by two steps of column chromatography and one of preparative PAGE as described by Browdy *et al.* (1990). Anti-Vt serum was prepared in rabbits after injection of 0.5 mg Vt as described by Tom *et al.* (1987b). The specificity of the antiserum was verified by immunoblotting (Bio-Rad immunoblot assay system) against all the fractions obtained during the purification as shown in Browdy *et al.* (1990). The antiserum reacted only with the Vt fraction.

Determination of oocyte diameter

Prior to *in vitro* incubations, a piece of tissue was removed from each ovary tested then fixed in 4% formalin in seawater. The average oocyte diameter was determined as described by Shlagman *et al.* (1986).

In vitro *incubation experiments*

Subepidermal adipose tissues (SAT's) were excised from females at different stages of vitellogenesis and from intermolt males. The SAT from each animal was divided into four pieces and each was incubated in Landureau's medium modified for penaeid tissues as described in Fainzilber *et al.* (1989). ^{14}C-leucine was added to a final concentration of 0.25 μCi/ml. Cold leucine level in the media was 0.11 m*M*.

Incubations were at 25°C with gentle shaking and an oxygen enriched atmosphere. Following the incubation period, tissues were removed and homogenized in 0.1 *M* phosphate buffer. Homogenates were centrifuged at 14,000 rpm for 10 min in an Eppendorf Microfuge at 4°C. *De novo* synthesized proteins were determined by TCA precipitation. Samples of tissue homogenates, supernatant, and the incubation media were pipetted onto Whatman 3 mm filter paper discs, dried, precipitated in 10% TCA, and washed according to Mans and Novelli (1961). Discs were counted in scintillation vials with 5 ml Aquasol-2 (New England Nuclear) in a Kontron Betamatic liquid scintillation counter, and quenching was monitored by measuring sample channels ratio (SCR). The specific activities of the media (cpm/μg leucine) were calculated from samples prepared on filter paper discs, and final results were calculated as μg leucine incorporated into TCA precipitable protein. Control incubations were carried out with media containing labelled leucine but lacking tissue and with tissue incubated without labelled leucine. Tissue protein was determined by the method of Bradford (1976).

Radioimmunoprecipitation (RIP) was carried out by taking 50 μl samples from tissue homogenates, supernatants and media. These samples were mixed with precalibrated volumes of anti-Vt serum after the addition of 0.1 mg purified Vt, to ensure maximum precipitation. Reaction volumes were adjusted to 1 ml with 0.4 *M* NaCl. Incubation was for 1 h at room temperature, after which vials were further incubated at 4°C for 24 h. Normal rabbit serum (NRS) was added instead of anti-Vt serum in control vials. Additional controls included NRS with a calibrated amount of goat-anti-rabbit serum, to obtain a large non-specific pellet. Following the incubation at 4°C, samples were centrifuged and the pellet washed three times with 0.4 *M* NaCl. Finally the pellet was dissolved in 75 μl of 0.5 *M* NaOH and incubated with vigorous shaking for 1 h at 37°C. The dissolved pellet was neutralized with 2 *M* HCl and the complete sample was applied to Whatman 3 mm paper discs, dried, and counted as described above.

The hepatopancreas was excised from previtellogenic and vitellogenic females and intermolt males, weighed, and each cut into 8–12 pieces. Each piece was weighed separately. They were prewashed for 1 h with frequent changes of media and each piece was incubated separately. Pieces of HEP were homogenized in their incubation media, centrifuged, and supernatants taken for analysis. Protease inhibitors (0.2 m*M* PMSF, 1 m*M* Aprotinin, 1 m*M* Leupeptin) were added, and the samples were analyzed as described above. All controls were as described for SAT incubations, with the addition of a zero time incubation control. Pieces of HEP were immersed in incubation media containing ^{14}C-leucine and were immediately homogenized and processed for analysis. Zero-time controls and background counts were similar. There was no difference between incubations with or without protease inhibitors. A number of different media compositions were tried and the best composition was found to be the modified Landureau medium buffered with Bis-Tris to pH 6.5.

All incubations and RIP were performed in culture plates or tubes coated with Sigmacote (Sigma Chemical Co.).

For fluorography from PAGE, incubations were as described above, except that media contained 2.5 μCi/ml ^{14}C-leucine and no cold leucine. Samples from these incubations were separated on PAGE. Gels were fixed in 7.5% acetic acid for 1 h, washed, and immersed in Amplify (Amersham, U.K.) for 30 min. Afterwards, gels were dried at 60°C under vacuum and exposed to Kodak XAR5 films at −70°C.

Collection of hemolymph and identification of hemocyanin

Hemolymph was removed from shrimp by cutting the rostrum and bleeding into 1 ml of a 10% sodium citrate solution. The hemolymphs of several females or several males were pooled and placed separately into test tubes. Hemocyanin was identified as the main constituent of whole hemolymph, which stained positively for copper (Horn and Kerr, 1963, 1969).

Results

Protein synthesis in in vitro *incubated SAT*

De novo protein synthesis and secretion to the medium were measured at regular time intervals during the incubation of the adipose tissues, and were found to increase linearly after an initial lag period of about 4 h, with a similar rate of protein synthesis in a male control (Fig. 1). Over 90% of the *de novo* synthesized TCA precipitable proteins were found in the medium fraction. A comparison of levels of total protein synthesis in SAT's of 13 females at different stages of vitellogenesis showed a large variability between females at the same vitellogenic stage, but failed to show any correlation between the vitellogenic

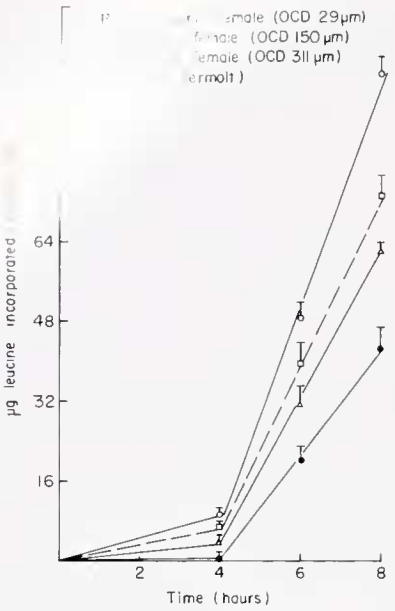

Figure 1. The incorporation rate of leucine into protein secreted by *in vitro* incubated subepidermal adipose tissue (SAT) in *Peneaus semisulcatus*. Tissues were removed from a male, and previtellogenic and vitellogenic females. Results were calculated for whole SAT. The mean ± S.D. for four replicates are given in each case. The average oocyte diameter (OCD) in ovaries is indicated (in μm) for each female.

stage and the synthesis of proteins in the adipose tissues (Table I). In excess antibody precipitations of all the samples, no Vt-specific proteins were detected. Ovaries from the same females all produced Vt *in vitro*, at levels averaging 38% of the total *de novo* synthesized protein (Browdy *et al.*, 1990; Table I).

SAT proteins were separated in non-denaturating polyacrylamide gels. Total proteins were visualized with Coomassie Blue (Fig. 2A), while incorporation of radiolabel into *de novo* synthesized SAT proteins was visualized by fluorography (Fig. 2B). Vt-specific immunological staining was performed on Western blots (Fig. 2C) and copper conjugated proteins by dithio-oxamide staining (Fig. 2D). Samples from previtellogenic and vitellogenic females and control males were run on each gel. Figure 2C shows that samples from SAT's of vitellogenic females stained positive for Vt. However, Vt was not visualized in the fluorographic exposures (Fig. 2B). One major radioactive band is seen in the fluorography of all the samples, including the male control. Based on the position of this band versus the position of hemocyanin (Fig. 2A), and on a positive reaction with the copper stain (Fig. 2D), the main *de novo* synthesized protein in adipose tissues appears to be hemocyanin or a copper containing protein with a similar electrophoretic mobility in non-denaturating polyacrylamide gels.

Protein synthesis in in vitro *incubated HEP*

Protein synthesis was measured in HEP incubated *in vitro* in a number of media and culture conditions, including those of Quackenbush (1989a, b). Protein synthesis in these cultures was low, and typically only 4–7% of the available label was incorporated into TCA precipitable proteins. This compares to 14–23% for ovarian

Table I

In vitro *protein synthesis in tissues of female (F) and male (M)* Penaeus semisulcatus

Tissue	Sex	Vitellogenic stage and oocyte diameter (μm)	Number of replicates[a]	Total protein synthesis[b]	Vt specific fraction[c] (%)
SAT	F	previtellogenic (≤100)	4	39.8 ± 15	0
	F	vitellogenic (100–300)	6	25.2 ± 9.2	0
	F	late vitellogenic (300–380)	3	28.2 ± 6.6	0
	M	—	4	26.6 ± 8.4	0
HEP	F	previtellogenic (≤100)	4	2.6 ± 1.3	5.2 ± 2.9
	F	vitellogenic (130–200)	4*	4.6 ± 1.6	14.4 ± 1.1
	F	vitellogenic (247–299)	5	0.4 ± 0.2	3.7 ± 2.0
	M	—	5	0.8 ± 0.3	0.8 ± 0.6
Ovary[d]	F	previtellogenic (≤100)	5	11.2 ± 3.1	5.0 ± 3.2
	F	vitellogenic (110–200)	4	55.2 ± 10.8	34.3 ± 4.7
	F	vitellogenic (220–281)	4	39.1 ± 4.5	42.3 ± 7.6

[a] Each replicate represents 2–3 pieces of tissue from a single animal, except where marked *. In this case, two pieces each from two animals were used.

[b] μg leucine incorporated into TCA precipitable protein per whole organ after 6 h of incubation (mean ± S.D.).

[c] Fraction of Vt specific immunoprecipitable protein from total *de novo* synthesized TCA precipitable protein (mean ± S.D.).

[d] Ovary data are based on Browdy *et al* (1990; Figs. 5, 6) and are shown for comparison.

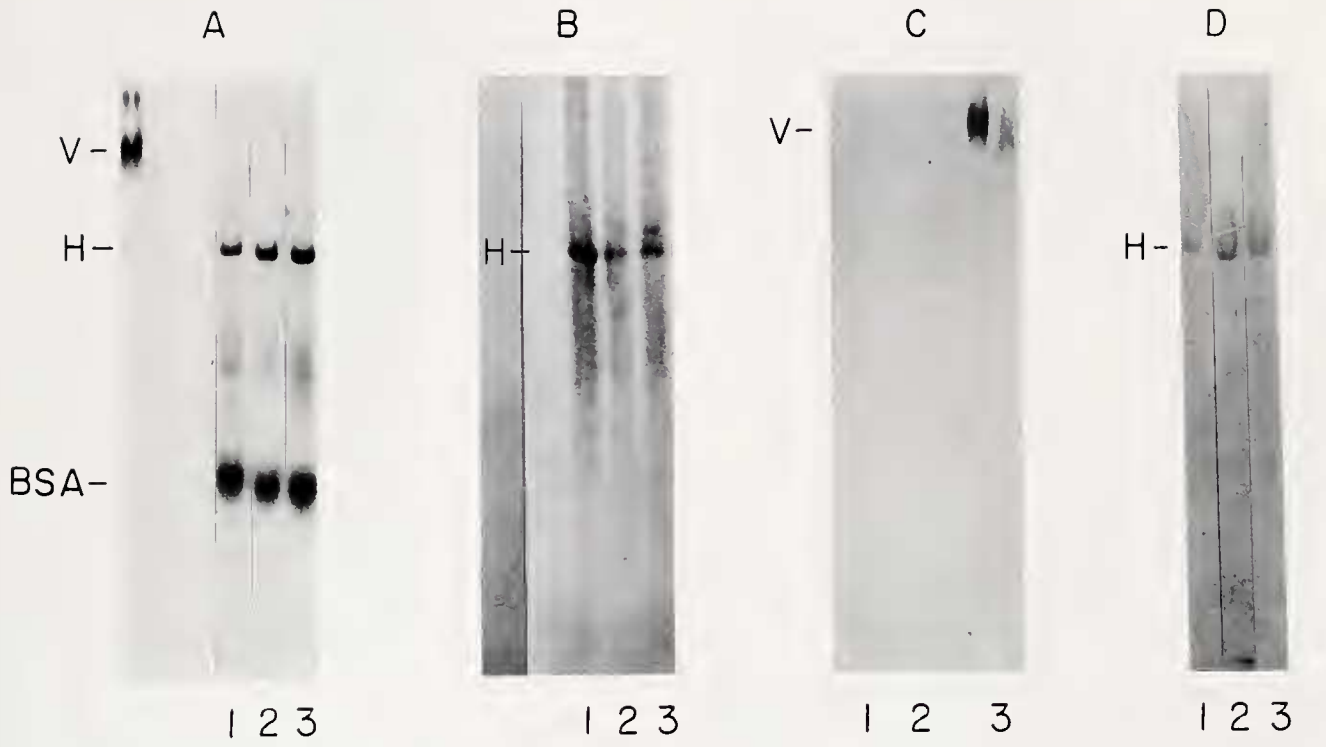

Figure 2. Non-denaturing polyacrylamide gel electrophoresis (5%) of protein synthesized *in vitro* by subepidermal adipose tissue (SAT) in *Peneaus semisulcatus*. Medium fractions only shown. (A) Coomassie blue stained gels. (B) Fluorography after 9 days of exposure. (C) Vt-specific immunological stain after Western blotting. (D) Copper-conjugated protein stain. Lanes: (1) male; (2) previtellogenic female (40 μm OCD); and (3) vitellogenic female (164 μm OCD). The positions of vitellin (V) and hemocyanin (H), which were run in separate lanes in the same gels, is indicated. Bovine serum albumin (BSA) was included in the culture media. Each lane in A, C, and D was run with 25 μl aliquots. In gels visualized by fluorography each lane was loaded with a sample containing 15,000–30,000 cpm of TCA precipitable protein.

cultures and 50–61% for SAT. The best conditions were found to be incubation in Landureau's medium modified for penaeid tissue (Fainzilber *et al.*, 1989) at pH 6.5 with 20 m*M* Bis-Tris. Protein synthesis rates in HEP from 11 females under these conditions differed according to the stage of vitellogenesis (Fig. 3, Table I), and were highest for females at an early stage of vitellogenesis (OCD 130–200 μm). Excess antibody precipitations revealed Vt-specific label ranging from 3.7% of total proteins synthesized in late vitellogenic females to 14.4% in early vitellogenesis (Table I). Male HEP cultures were also found to contain Vt-specific label, at near-background levels of 0.8%.

Comparison between different tissues

To evaluate the possible quantitative significance of the Vt synthesis in HEP cultures, leucine incorporation results were translated into Vt synthesis rates, calculated from the molar ratio of leucine in purified *Penaeus semisulcatus* Vt (Tom *et al.*, in press). These calculated Vt synthesis values were corrected to allow for the 2 h lag in protein synthesis observed in HEP incubations of early vitellogenic females (Fig. 3), and the final values obtained represent synthesis rates during the linear phase. Similar calculations were performed for ovaries incubated *in vitro*, from the data of Browdy *et al.* (1990). Ovarian Vt synthesis rates ranged from 1.2 μg Vt/whole ovary/h in previtellogenic females to 36.4 μg Vt/whole ovary/h in vitellogenic females (Fig. 4). These values were 3–23 times higher than the corresponding Vt synthesis rates in HEP cultures (0.34–1.6 μg Vt/whole HEP/h in previtellogenic and early vitellogenic females, Fig. 4).

Fluorography of TCA precipitates from female HEP samples run on polyacrylamide gels failed to show radioactivity in the region of the Vt band, which is clearly seen in the parallel lane of an ovary sample (Fig. 5). One of the main bands seen in fluorography of samples from HEP incubations has the same position as does the putative hemocyanin band from SAT samples (Fig. 5).

Discussion

The results shown above and those of Browdy *et al.* (1990) strongly suggest that the ovary is the main site of

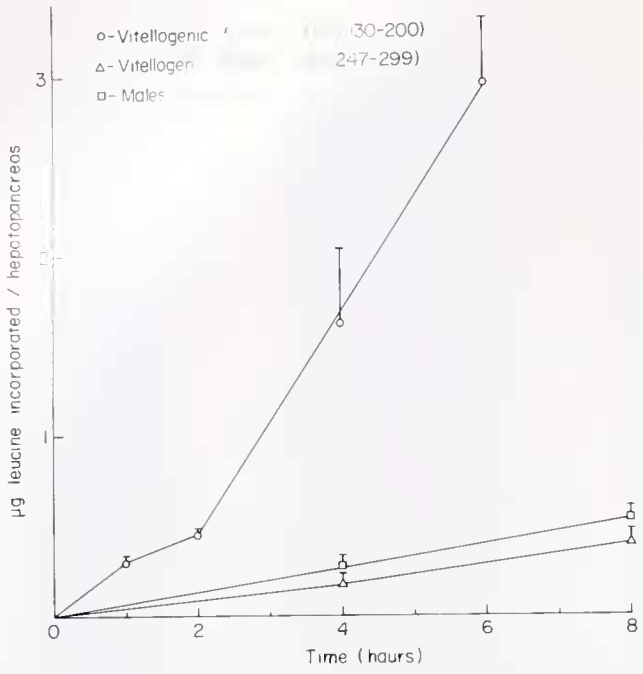

Figure 3. The incorporation rate of leucine into protein by *in vitro* incubated hepatopancreas pieces in *Peneaus semisulcatus*. Results (mean ± S.D.) were calculated per whole hepatopancreas and are presented for males and early and late vitellogenic females. Mean ± S.D. for four replicates at each time point are shown. The average oocyte diameters (OCD) in the ovaries of the females are indicated in µm.

Vt synthesis in *Penaeus semisulcatus* and that the SAT has no role in this process. These conclusions are based on Vt-specific immunoprecipitations (Table I) and fluorography of samples on PAGE (Fig. 2). The main protein synthesized in *in vitro* incubations of SAT appears to be hemocyanin or a related protein based on its positive copper stain and position in PAGE (Fig. 2). The fact that the SAT-synthesized protein has the same position in PAGE as the main HEP product (Fig. 5) is noteworthy, since the HEP is a well characterized hemocyanin synthesizing tissue in decapod crustaceans (Senkbeil and Wriston, 1981; Bielefeld *et al.*, 1986; Preaux *et al.*, 1986). There are no previous reports of hemocyanin synthesis in adipose tissues of Crustacea.

Hemocyanin accounts for 90–95% of the hemolymph serum proteins in crustaceans (Horn and Kerr, 1969; Rochu and Fine, 1978; Ferrero *et al.*, 1983; Hagerman, 1983; de Fur *et al.*, 1985). Most of the copper found in the hemolymph (about 95%) was found to be bound to hemocyanin (Horn and Kerr, 1963). Evidence in the literature strongly suggests the midgut gland (HEP) as a site of synthesis of hemocyanin in several crustacean species (see review of Mangum, 1983). However, Ghiretti-Magaldi *et al.* (1977) postulated a "lymphocytogluic" organ associated with the gizzard, and Senkbeil and Wriston (1981) observed synthesis on the "intestinal wall". The high levels of synthesis found in the present report suggest that the SAT may be a major hemocyanin synthesizing tissue in penaeid shrimp, and should motivate additional research into the role of adipose tissues in this process.

The quantitative significance of the HEP in Vg synthesis in penaeid shrimp is still unclear. The data shown above (Table I, Fig. 4), suggest that this tissue synthesizes a Vt-immunoreactive protein in previtellogenic and early vitellogenic females. However, it seems that quantitatively the contribution of the HEP is low. Previous studies of protein synthesis *in vitro* in HEP of *Penaeus japonicus* and *Penaeus vannamei* have reported conflicting results. Yano and Chinzei (1987) did not detect synthesis of Vt-specific proteins in *in vitro* incubated HEP of *P. japonicus*. However, their incubations were performed in salts ringer without buffer, vitamins and sugar supplements, that we have found to be essential for cultures of HEP, and the total protein synthesis they reported was very low. Rankin *et al.* (1989) reported that incorporation of radiolabelled leucine in *in vitro* incubated HEP of *P. vannamei* was relatively high early in the cycle of ovarian development. They did not observe changes in the SDS-PAGE peptide pattern during ovarian development, and did not use antibodies or other means of identifying Vg specific synthesis. The synthesis of yolk proteins reported by

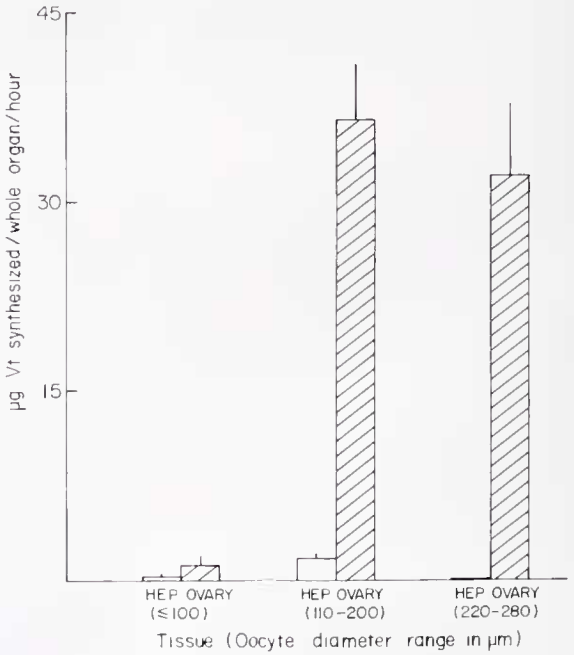

Figure 4. Vitellin synthesis rates in hepatopancreases (HEP) versus ovaries incubated *in vitro*, in females of *Peneaus semisulcatus* at different stages of vitellogenesis. Ovary data are calculated from the results of Browdy *et al.* (1990). Synthesis rates are calculated for the whole organ, and represent the linear phase of protein synthesis, after the lag period (see Fig. 1).

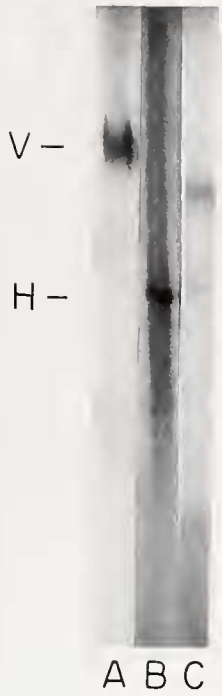

Figure 5. Comparative fluorography of proteins synthesized *de novo* in the ovary (A), subepidermal adipose tissue (B), and hepatopancreas (C), during *in vitro* incubations of tissues from vitellogenic females of *Penaeus semisulcatus*. The positions of vitellin (V) and hemocyanin (H) are shown. 15,000 cpm of TCA precipitable proteins were loaded in each lane.

Quackenbush (1989a, b) for HEP of *Penaeus vanammei* is not clearly attributable to Vg synthesis. This is due to the use of an antigen for antibody production which does not represent the whole Vt. This antigen was a 97 kD protein resolved from SDS-PAGE (and therefore in denaturated form) and found in both ovaries and HEP. The antibody to this antigen reacted with three different ovarian proteins and one hepatopancreatic protein. The levels of immunoreactive proteins in ovaries of *P. vannamei*, when measured with this antibody, reached a maximum level of 8% of total tissue protein (Quackenbush, 1989b), much lower than the expected levels for the main storage protein of a mature penaeid ovary (Rankin *et al.*, 1989).

Our comparison of the Vt synthesis rates per whole organ in HEP versus ovary (Fig. 4) clearly shows that there is a difference of one order of magnitude in Vt synthesis rates in these two organs incubated *in vitro*. We cannot rule out the possibility that this difference is less extreme *in vivo*. However, Vg hemolymph levels and turnover were found to be low in both previtellogenic and vitellogenic *P. semisulcatus* females (Shafir *et al.*, 1992 and unpub. obs.). These results lead us to the conclusion that although the HEP may be involved in the synthesis of some ovarian proteins, its quantitative significance is probably low. The possibility that the HEP may be involved in synthesis of an exclusively extraovarian Vt component has yet to be shown.

Vitellin-specific immunoreactivity has been found in SAT samples of *Penaeus semisulcatus* (Fig. 2C), *P. japonicus* (Vazquez-Boucard, 1985) and *Parapenaeus longirostris* (Tom *et al.*, 1987a). Vitellin-positive reactions have also been observed in the immunohistological preparations of HEP from two brachyuran decapods (Paulus and Laufer, 1987). These results show the presence of Vt immunoreactive compounds, but do not provide evidence for active synthesis of these compounds in the tissues examined. A high molecular weight Vt immunoreactive compound has been found in the hemolymph of several crustaceans. The Vt positive reactions in tissues may be due to absorption of hemolymph in the incubated tissues, or may suggest a role for these tissues in the catabolism of Vt following resorption of oocytes. Oocyte resorption provides one of the explanations for the presence of Vt immunoreactivity in the hemolymph. It is also possible that such immunoreactivity results from Vg synthesis in ovarian follicle cells, secretion of the Vg into the hemolymph, and subsequent uptake into developing oocytes (Yano and Chinzei, 1987).

It should be noted that most of the data supporting the ovary as a single or main site of Vt synthesis in penaeids have been obtained from *in vitro* systems. Such systems, although possessing many advantages for subsequent endocrinological studies, might not provide the necessary conditions for a specific tissue to function as it does *in vivo*. Conversely, an *in vivo* experimental approach does not allow the clear isolation of a specific tissue's contribution. Identification of yolk protein mRNA from tissues of females at different stages of vitellogenesis, using *in vitro* translation systems could clarify this issue. These techniques have been used with success for the study of hemocyanin synthesis in crayfish (Bielefeld *et al.*, 1986). The feasibility of this approach is currently under examination in our laboratory.

To summarize, our results and other recent publications suggest that the ovary is the most prominent site for Vt synthesis in penaeid shrimp. This is in contrast to the extraovarian synthesis of Vg in Isopoda and Amphipoda (Meusy and Payen, 1988). It seems that different groups of crustaceans use different strategies for the synthesis of their yolk proteins. In this respect, crustaceans are apparently similar to insects, in which at least three different strategies of yolk protein synthesis have been characterized (Kanost *et al.*, 1990). It is noteworthy that insects with different strategies of yolk synthesis also differ in various aspects of the endocrine regulation of this process, a fact with interesting implications for future studies of endocrine regulation of vitellogenesis in crustaceans.

Acknowledgments

This research was supported by grants from the Israeli Ministry of Energy and Infrastructure, the Binational Agricultural Research and Development Fund (Project No. I-147-[illegible]), and the Basic Research Fund of the Israeli National Academy of Sciences (Project No. 231/91). We thank Dr. Craig Browdy for helpful discussions, and two anonymous reviewers for their constructive criticism.

Literature Cited

Adiyodi, R. G., and T. Subramoniam. 1983. Oogenesis, Oviposition, and Oosorption. Pp. 443–495. In *Reproductive Biology of Invertebrates,* Vol. 1., K. G. Adiyodi and R. G. Adiyodi, eds. Wiley, New York.

Bielefeld, M., G. Gellissen, and K. D. Spindler. 1986. Protein production and the moulting cycle in the crayfish *Astacus leptodactylus. Insect Biochem.* **16:** 175–180.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72:** 248–254.

Browdy, C. L., and T. M. Samocha. 1985. The effect of eyestalk ablation on spawning, molting and mating of *Penaeus semisulcatus* de Haan. *Aquaculture* **49:** 19–29.

Brnwdy, C. L., M. Fainzilber, M. Tom, Y. Loya, and E. Lubzens. 1990. Vitellin synthesis in relation to oogenesis in *in vitro* incubated ovaries of *Penaeus semisulcatus* (Crustacea, Decapoda, Penaeidae). *J Exp. Zool.* **255:** 205–215.

Caubere, J. L., R. Lafon, F. Rene, and C. Sales. 1976. Maturation et ponte chez *Penaeus japonicus* en captivite, essai de controle de cette reproduction a Maguelonne dans les cotês Françaises. FAO Tech. Conf. on Aquaculture, Kyoto, Japan. FIR:AQ/Conf/76/E.49. 18 pp.

Ceccaldi, H. J. 1970. Evolution des proteines de l'hemolymphe de *Penaeus kerathurus* femelle durant la vitellogenese. *C.R. Seances Soc. Biol. Fil.* **164:** 2572–2575.

Charniaux-Cotton, H., and G. Payen. 1988. Crustacean reproduction. Pp. 279–303 in *Endocrinology of Selected Invertebrate Types,* vol. 2. H. Laufer, and R. G. H. Downer, eds. Alan R. Liss Inc., New York.

Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121:** 404–427.

De Fur, P. L., C. P. Mangum, and B. R. McMahon. 1985. Cardiovascular and ventilatory changes during ecdysis in the blue crab *Callinectes sapidus* Rathbun. *J. Crust. Biol.* **5:** 207–215.

Dehn, P. F., D. E. Aiken, and S. L. Waddy. 1983. Aspects of vitellogenesis in the lobster *Homarus americanus. Can. Tech. Rep. Fish. Aquat. Sci.* **1161:** iv + 24 pp.

Eastman-Reks, S., and M. Fingerman. 1985. *In vitro* synthesis of vitellin by the ovary of the fiddler crab *Uca pugilator. J Exp. Zool.* **233:** 111–116.

Fainzilber, M., C. L. Browdy, M. Tom, E. Lubzens, and S. W. Applebaum. 1989. Protein synthesis *in vitro* in cultures of the subepidermal adipose tissue and the ovary of the shrimp *Penaeus semisulcatus. Tissue Cell* **21:** 911–916.

Ferrero, E. A., G. Graziosi, R. Marzari, and A. Mosco. 1983. Protein pattern variability of the hemolymph of mantis shrimp, *Squilla mantis* L. (Crustacea, Stomatopoda). *J. Exp. Zool.* **225:** 341–345.

Fingerman, M. 1987. The endocrine mechanisms of crustaceans. *J. Crust. Biol.* **7:** 1–24.

Fyffe, W. E., and J. D. O'Connor. 1974. Characterization and quantification of a crustacean lipovitellin. *Comp Biochem. Physiol.* **47B:** 851–867.

Ghiretti-Magaldi, A., C. Milanesi, and G. Tognon. 1977. Hemopoiesis in Crustacea Decapoda: origin and evolution of hemocytes and cyanocytes of *Carcinus maenas. Cell Diff.* **6:** 167–186.

Gohar, M., C. Souty-Grusset, and R. Juchault. 1985. Rhythme nycthemeral de la synthese proteique ovarienne chez le crustacé oniscoide *Porcellio dilatatus* Brandt et rôle du protocerebron. *Can. J. Zool.* **63:** 799–803.

Hagerman, L. 1983. Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. *Mar. Biol.* **77:** 11–17.

Hames, B. D., and D. Rickwood. 1981. *Gel Electrophoresis of Proteins. A Practical Approach* IRL Press, Oxford, 290 pp.

Horn, E. C., and M. S. Kerr. 1963. Hemolymph protein and copper concentrations of adult blue crabs (*Callinectes sapidus* Rathbun). *Biol. Bull.* **125:** 499–507.

Horn, E. C., and M. S. Kerr. 1969. The hemolymph proteins of the blue crab, *Callinectes sapidus.* I. Hemocyanins and certain other major protein constituents. *Comp. Biochem. Physiol.* **29:** 493–508.

Huang, E. S., K. J. Kao, and A. V. Nalbandov. 1979. Synthesis of sex steroids by cellular components of chicken follicles. *Biol. Reprod.* **20:** 454–461.

Junera, H., and J. J. Meusy. 1982. Vitellogenin and lipovitellins in *Orchestia gammarellus* (Pallas) (Crustacea, Amphipoda); labelling of subunits after *in vivo* administration of ^{3}H-leucine. *Experientia* **38:** 252–253.

Kanost, M. R., J. K. Kawooya, J. H. Law, R. O. Ryan, M. C. Van Heusden, and R. Ziegler. 1990. Insect haemolymph proteins. Pp. 299–396 in *Advances in Insect Physiology,* vol. 22. Academic Press, New York.

Kerr, M. S. 1968. Protein synthesis by the hemocytes of *Callinectes sapidus:* a study of *in vitro* incorporation of ^{14}C-leucine. *J Cell. Biol.* **39:** 72a–73a.

Lam, T. J., S. Pandey, Y. Nagahama, and W. S. Hoar. 1978. Endocrine control of oogenesis, ovulation and oviposition in goldfish. Pp. 55–64 in *Comparative Endocrinology.* P. J. Gaillard, and H. H. Boer, eds. Elsevier, Amsterdam.

Laufer, H., and R. G. H. Downer. 1988. *Endocrinology of Selected Invertebrate Types.* Alan R. Liss Inc., New York, 500 pp.

Lui, C. W., and J. D. O'Connor. 1976. Biosynthesis of lipovitellin by the crustacean ovary. II. Characterization and *in vitro* incorporation of amino acids into the purified subunits. *J Exp. Zool.* **195:** 41–52.

Mangum, C. P. 1983. Oxygen transport in the blood. Pp. 373–429 in *The Biology of Crustacea,* vol. 5. D. E. Bliss, ed. Academic Press, New York.

Mans, R. J., and G. D. Novelli. 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter paper disk method. *Arch Biochem. Biophys.* **94:** 48–53.

Marzari, R., E. Ferrero, A. Mosco, and A. Savoini. 1986. Immunological characterization of the vitellogenic proteins in *Squilla mantis* hemolymph (Crustacea, Stomatopoda). *Exp. Biol.* **45:** 75–80.

Meusy, J. J. 1980. Vitellogenin, the extraovarian precursor of the protein yolk in Crustacea: a review. *Reprod Nutr. Develop.* **20:** 1–21.

Meusy, J. J., and G. G. Payen. 1988. Female reproduction in malacostracan Crustacea. *Zool. Sci.* **5:** 217–265.

Nelson, K., B. Heyer, E. Johnson, D. Hedgecock, and E. S. Chang. 1988. Photoperiod-induced changes in hemolymph vitellogenins in female lobsters (*Homarus americanus*). *Comp. Biochem. Physiol.* **90B:** 809–821.

Paulus, J. E., and H. Laufer. 1987. Vitellogenocytes in the hepatopancreas of *Carcinus maenas* and *Libinia emarginata* (Decapoda Brachyura). *Int. J. Invertebr. Reprod. Develop.* **11:** 29–44.

Picaud, J. L. 1980. Vitellogenin synthesis by the fat body of *Porcellio dilatatus* Brandt (Crustacea, Isopoda). *Int. J. Invertebr. Reprod.* **2:** 347–349.

Preaux, G., A. Vandamme, B. de Bethune, M. P. Jacobs, and R. Lontie. 1986. Hemocyanin-mRNA-rich fractions of Cephalopodan Decabranchia and of Crustacea, their *in vivo* and *in vitro* translation. Pp. 485–488 in *Invertebrate Oxygen Carriers.* B. Linzen, ed. Springer-Verlag, Berlin.

Quackenbush, L. S. 1986. Crustacean endocrinology, a review. *Can. J. Fish. Aquat. Sci.* **43:** 2271–2282.

Quackenbush, L. S. 1989a. Vitellogenesis in the shrimp, *Penaeus vannamei: in vitro* studies of the isolated hepatopancreas and ovary. *Comp. Biochem. Physiol.* **94B:** 253–261.

Quackenbush, L. S. 1989b. Yolk protein production in the marine shrimp *Penaeus vannamei. J. Crust. Biol.* **9:** 509–516.

Quackenbush, L. S., and L. L. Keeley. 1988. Regulation of vitellogenesis in the fiddler crab, *Uca pugilator. Biol. Bull.* **175:** 321–331.

Rankin, S. M., J. Y. Bradfield, and L. L. Keeley. 1989. Ovarian protein synthesis in the South American white shrimp, *Penaeus vannamei,* during the reproductive cycle. *Invertebr. Reprod. Dev.* **15:** 27–33.

Rochu, D., and J. M. Fine. 1978. Antigenic structure of the hemocyanin in six species of decapod Crustacea. *Comp. Biochem Physiol.* **59A:** 145–150.

Senkbeil, E. G., and J. C. Wriston. 1981. Hemocyanin synthesis in the American lobster *Homarus americanus. Comp. Biochem. Physiol.* **68B:** 163–171.

Shafir, S., M. Tom, M. Ovadia, and E. Lubzens. 1992. Protein, vitellogenin and vitellin levels in the haemolymph and ovaries during ovarian development in *Penaeus semisulcatus* (de Haan). *Biol. Bull.* **183:** (in press).

Shlagman, A., C. Lewinsohn, and M. Tom. 1986. Aspects of the reproductive activity of *Penaeus semisulcatus* de Haan along the southeastern coast of the Mediterranean. *Mar. Ecol.* **7:** 15–22.

Souty, C., and J. L. Picaud. 1981. Vitellogenin synthesis in the fat body of the marine crustacean Isopoda *Idotea balthica baoteri* during vitellogenesis. *Reprod. Nutr. Dev.* **21:** 95–101.

Suzuki, S. 1987. Vitellins and vitellogenins of the terrestrial isopod, *Armadillidium vulgare. Biol. Bull.* **173:** 345–354.

Tata, J. R. 1976. The expression of the vitellogenin gene. *Cell* **9:** 1–14.

Tom, M., M. Goren, and M. Ovadia. 1987a. Localization of the vitellin and its possible precursors in various organs of *Parapenaeus longirostris* (Crustacea, Decapoda, Penaeidae). *Int. J. Invertebr. Reprod. Dev.* **12:** 1–12.

Tom, M., M. Goren, and M. Ovadia. 1987b. Purification and partial characterization of vitellin from the ovaries of *Parapenaeus longirostris* (Crustacea, Decapoda, Penaeidae). *Comp. Biochem. Physiol.* **87B:** 17–23.

Tom, M., M. Fingerman, T. K. Hayes, V. Johnson, B. Kerner, and E. Lubzens. in press. A comparative study of the ovarian proteins from two penaeid shrimps, *Penaeus semisulcatus* (De Haan) and *Penaeus vannamei* (Boone). *Comp. Biochem. Physiol.*

Vazquez-Boucard, C. 1985. Identification preliminaire du tissu adipeux chez le crustacé Decapode *Peneus japonicus* Bate, a l'aide de anticorps antilipoistellene. *C.R. Acad. Sci Ser. III Sci. Vie* **300:** 95–97.

Wolin, E. M., H. Laufer, and D. F. Albertini. 1973. Uptake of the yolk protein, lipovitellin, by developing crustacean oocytes. *Dev. Biol.* **35:** 160–170.

Yano, I., and Y. Chinzei. 1987. Ovary is the site of vitellogenin synthesis in kuruma prawn, *Penaeus japonicus. Comp. Biochem. Physiol.* **86B:** 213–218.